REMARKS

The Examiner rejected Claim 1 as being unpatentable over Reuser *et al.* (US Patent 6118045), Van Hove *et al.* (Proc. Natl. Acad. Sci. 93:65-70 (1996)), and Kikuchi *et al.* (J. Clin. Invest. 101(4):827-833 (1998)). The teachings of each of these three references are addressed individually below, followed by a discussion of the combination of the references, as well as an assessment of the state of the art regarding Pompe disease at the time this application was filed.

Reuser *et al.*

Reuser *et al.* describe transgenic nonhuman animals producing acid alpha-glucosidase in milk. Although Reuser *et al.* state that recombinant lysosomal proteins "find use in enzyme replacement therapeutic procedures" (Col. 10, lines 64-65), they do not, in fact, describe treatment of a human patient, nor do they describe biweekly administration of a therapeutically effective dose to a human patient.

The experiments of Reuser *et al.* do indicate that active enzymes were taken up by fibroblasts from Pompe patients (col. 15, lines 37-65). It is well known in the art, however, that *in vitro* cell culture conditions differ significantly from *in vivo* conditions: for example, when enzyme is administered *in vivo* by intravenous infusion, the muscle cells don't come into direct contact with enzyme as they do in cell culture. Furthermore, the endothelial barrier, as well as the interstitial connective tissue, must be passed *in vivo*, such as described by Reuser *et al. (Eur. J. Pediatr.* 161:S106-S111 (2002); a copy of which was submitted as an Exhibit previously). The lack of correlation between *in vitro* fibroblast experiments and *in vivo* results is further emphasized in the report at the 53rd Annual Meeting of the American Society of Human Genetics in November 2003, where it is stated:

> The results obtained from in vitro fibroblast uptake studies (see Poster #2691) do not predict *in vivo* efficacy, as shown here. This illustrates the significant impact of competing mechanisms of uptake present in a multi-cellular system.

For the convenience of the Examiner, a copy of the cited Poster is attached as Exhibit 1; see the Conclusions.

Van Hove *et al.*

Van Hove *et al.* describe purification methods for production of recombinant acid alpha-glucosidase produced in Chinese hamster ovary (CHO) cells. Van Hove *et al.* indicate that intravenous injection of enzyme into a non-human animal (a guinea pig) increased enzyme levels in liver and heart (pp. 613-614). There is no indication that the increased enzyme levels *in vivo* would result in reduction of accumulated glycogen or arresting of accumulation of glycogen. Thus, as with Reuser *et al.*, Van Hove *et al.* do not describe administration of acid alpha-glucosidase to a human being, let alone treatment of a human patient with Pompe's disease.

Kikuchi *et al.*

Kikuchi *et al.* describe administration of recombinant human acid alpha-glucosidase to acid maltase-deficient quail, and resultant increase in enzyme activity and decrease in glycogen levels in heart and liver, and essentially normal morphology in pectoralis muscle except for increased glycogen granules. Kikuchi *et al.* do not describe treatment of a human patient; in addition, the quail were injected seven times over a sixteen day period (p. 828, second column), and not biweekly as indicated in the pending claim.

It should be noted that there are significant structural difference in mannose-6-phosphate receptors between birds and mammals which limit the utility of this quail model for studying mannose-6-phosphate mediated enzyme replacement therapy. See, for example, Yang, H.W. *et al.*, cited previously as reference C20 in an Information Disclosure Statement. In view of these significant differences, the results set forth by Kikuchi *et al.* do not reasonably predict that treatment of a human patient with Pompe's disease by biweekly intravenous administration of acid alpha-glucosidase would be successful.

Combination of the References

The Examiner states that the references "clearly demonstrate to one in the art that IV administration of alpha-glucosidase to a patient with Pompe's disease is an effective treatment which results in reduced glycogen accumulation as well as arresting further accumulation of glycogen" (Office Action, p. 4, first full paragraph). Applicants respectfully disagree. None of the references, either alone or in combination, indicate that glycogen accumulation in a human

patient can be reduced or arrested. In fact, the Kikuchi *et al.* reference specifically states that "[c]urrently, there is no effective treatment for Pompe disease" (p. 827, first full paragraph). In view of this statement by Kikuchi *et al.* (published in 1998), one of ordinary skill in the art would not have assumed that the teachings of Reuser *et al.*(filed in 1996) or Van Hove *et al.* (published in 1996) were at all indicative of the existence of an effective treatment for the disease.

As discussed above, reduction of glycogen accumulation in fibroblasts *in vitro* is not indicative of efficacy of treatment *in vivo,* in view of the significant differences between *in vitro* and *in vivo* conditions and availability of enzyme to cells; furthermore, the *in* vivo experiments of Van Hove *et al.* and Kikuchi *et al.* either do not indicate that reduction of glycogen accumulation occurs, or indicates that such reduction occurs in a non-mammalian animal with the use of multiple (more than biweekly) treatments. In view of these considerations, one of ordinary skill in the art would not have had a reasonable expectation of successfully treating a human individual with Pompe's disease.

State of the Art at the Time the Application was Filed

At the time Applicants' invention was made, Pompe's disease had been known for about seventy years, since the early 1930's, and the enzyme deficiency causing the disease had been known for about forty years, since the 1960's. Despite the length of time the disease has been known, numerous attempts at treatment by administration of replacement enzyme had failed. For example, Van der Ploeg *et al.* (*J. Clin. Invest* 87:513-518 (1991), cited in IDS as reference C14) lists several references that indicate that attempts at enzyme replacement therapy had failed. See also Williams *et al.*, Birth Defects, Original Article Series, Vol. XVI, No. 1, pages 415-423 (1980). Williams *et al.* describe administration of two doses of enzyme extracted from human liver and then linked to low density lipoprotein (LDL). Williams *et al.* concluded that their experiments were failures. For example, on page 420, they state:

> A quadriceps muscle biopsy was performed 2 days after the infusion and the glycogen content was not significantly altered. No α- glucosidase activity against glycogen was detected.

They also state that "The slight decrease in glycogen content of tissues is of questionable significance" (p. 422) and note that the patient died 26.5 days after the second infusion (p. 420).

Another reference describing failure of enzyme therapy is set forth by de Barsy *et al.* (Birth Defects, Original Article Series, Vol. IX, No. 2, pages 184-190 (1973)), who describe extraction of GAA from human placenta, and administration of a single dose of the GAA to an infant. de Barsy *et al.* report that "no conspicuous morphologic or clinical improvements were noted" in the patient.

Without treatment as claimed in the instant application, children afflicted with the disease are expected to die, and most untreated infants with the disease are not expected to live beyond two years of age.

In view of these multiple failures known in the art, it is evident that at the time the invention was made, there was a long-felt need but no reasonable expectation of a successful treatment. As stated in Boehrenger Ingelheim Vetmedica, Inc. v. Schering-Plough Corp., 320 F.3d 1339, 1354, 65 U.S. P.Q. 2d 1961, 1972 (Fed. Cir. 2003), "...there can be little better evidence negating an expectation of success than actual reports of failure."

In this context of the long-felt need for a treatment and the failure of others to treat Pompe's disease successfully, including attempts to treat the disease by enzyme replacement, one skilled in the art would not have had any reasonable expectation of success in treating human individuals with Pompe's disease using acid alpha-glucosidase. In spite of this bleak prospect, Applicants have, in fact, succeeded in treating patients with Pompe's disease, as evidenced by successful clinical trials that have led to the approval by the Food and Drug Administration of the product, Myozyme®, for the treatment of Pompe's disease. Representative news articles describing the near-miraculous improvement of many Pompe disease patients were attached as Exhibits B, C and D to the Amendment filed previously. This incredible success in view of the long-felt need and failures of others further evidences that the claimed invention is non-obvious over the teachings of the cited references under 35 U.S.C. 103(a).

The Supreme Court, in *KSR Int'l v. Teleflex, Inc*, (No. 04-1350), held that courts must investigate whether a claimed improvement in an art is more than predictable:

> The principle underlying these cases are instructive when the question is whether a patent claiming the combination of elements of prior art is obvious. When a work is available in one field of endeavor, design incentives and other market forces can prompt variations of it, either in the same field or in a different one. If a person of ordinary skill in the art can implement a predictable

> variation, § 103 likely bars its patentability. For the same reason, if a technique has been used to improve one device, and a person of ordinary skill in the art would recognize that it would improve similar devices in the same way, using the technique is obvious unless its actual application is beyond his or her skill. Sakraida and Anderson's Black Rock are illustrative—a court must ask whether the improvement is more than the predictable use of prior art elements according to their established functions.

Id. at 13.

Moreover, the *KSR* Court reiterated the broad base from which teachings can be drawn, and the flexible nature of the statutory requirement of non-obviousness:

> Helpful insights, however, need not become rigid and mandatory formulas; and when it is so applied the TSM [teaching-suggestion-motivation] test is incompatible with our precedent. The obviousness analysis cannot be confined by a formulistic conception of the words teaching, suggestion, and motivation; or by overemphasis on the importance of published articles and the explicit content of issued patents. The diversity of inventive pursuits and of modern technology counsels against limiting the analysis this way. In many fields it may be that there is little discussion of obvious techniques or combinations, and it often may be the case that market demand, rather than scientific literature, will drive design trends. Granting patent protection to advances that would occur in the ordinary course without real innovation retards progress and may, in the case of patents combining previously known elements, deprive prior inventions of their value or utility.

Id. at 15.

In light of the decision in *KSR*, Claim 1 of the present application is patentable over the cited references, as the claimed invention is more than the predictable use of prior art elements according to their established functions, particularly in view of the long-established lack of success in the art, and the absence of any indication of the utility of biweekly intravenous administration of the enzyme as claimed. In addition, the combination of the references does not render Claim 1 obvious as the cited references do not disclose or suggest the subject matter of Claim 1.

CONCLUSION

In view of the above amendments and remarks, it is believed that Claim 1 is in condition for allowance, and it is respectfully requested that the application be passed to issue. If the Examiner feels that a telephone conference would expedite prosecution of this case, the Examiner is encouraged to call the undersigned.

Respectfully submitted,

HAMILTON, BROOK, SMITH & REYNOLDS, P.C.

David E. Brook, R.N. 22592

By for Elizabeth W. Mata

Elizabeth W. Mata
Registration No. 38,236
Telephone: (978) 341-0036
Facsimile: (978) 341-0136

Concord, MA 01742-9133

Date: 8/29/07

EXHIBIT 1

An *In Vivo* Comparison of the Efficacy of Acid α-Glucosidase Produced in CHO Cells and Transgenic Rabbits

Alison McVie-Wylie, Cindy Rogers, Russell Gotschall, Beth Thurberg, William Canfield, Robert Mattaliano and Laura Andrews
Genzyme Corporation, Framingham, MA

Introduction

Pompe disease (also known as Glycogen Storage Disease Type II or Acid Maltase Deficiency) is an autosomal recessive disorder of glycogen metabolism resulting from the deficiency of the lysosomal enzyme acid α-glycosidase (GAA). The lack of this enzyme results in massive lysosomal glycogen accumulation primarily in the cardiac and skeletal muscles. The clinical manifestations of Pompe disease range from a severe, infantile form characterized by cardiomyopathy and skeletal muscle weakness, to a slowly progressive adult presentation limited to skeletal muscle.

Enzyme replacement therapy (ERT) has been developed for Pompe disease and early phase clinical trials have been conducted, or are ongoing, using recombinant human GAA purified from the milk of transgenic rabbits (tgGAA) and from genetically modified CHO cells (rhGAA). In independent laboratory studies, both enzymes have demonstrated efficacy in the Pompe mouse animal model; however, for direct comparison, a study was designed to determine the relative efficacy under the same experimental conditions.

Study Design

Pompe mice (N. Rabin, NIH) were administered either tgGAA or one of two rhGAA preparations intravenously (via tail vein) for 4 weekly doses.

Three dose levels were investigated for each enzyme:

- 20, 60 and 100mg/kg

All injected animals were sacrificed 24 hours after the last dose with the heart, diaphragm, quadriceps, triceps and psoas muscle collected for glycogen analysis, enzyme activity and Western blotting.

Results

Glycogen Clearance

Glycogen content was determined in selected tissues using both a biochemical method and a histological method (Metamorph).

The results demonstrated that, under the conditions of the study, a dose dependant decrease in tissue glycogen content was seen with all enzyme preparations tested. However, the reduction of glycogen following treatment with tgGAA was less than that observed with either of the rhGAA preparations produced in CHO cells.

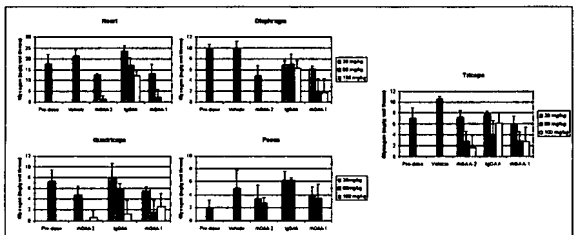

Figure 1:
Biochemical glycogen analysis of selected tissues from Pompe mice treated with tgGAA or one of two rhGAA preparations

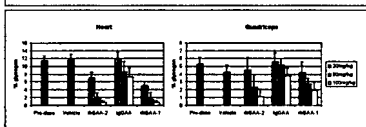

Figure 2:
Metamorph glycogen analysis of selected tissues from Pompe mice treated with tgGAA or one of two rhGAA preparations

GAA Activity Analysis

GAA enzyme activity analysis of selected tissues was determined using a 4MU-glucoside fluorometric assay.

Interestingly, the results indicated that higher levels of enzyme were present in the tissues of mice treated with tgGAA compared to the levels detected in the tissues of the rhGAA treated mice, despite the finding of less glycogen clearance in these tissues. This suggests that tgGAA may be preferentially targeting to cell types other than glycogen-containing myocytes within these tissues, or is less efficient at targeting to the lysosomes within the myocyte.

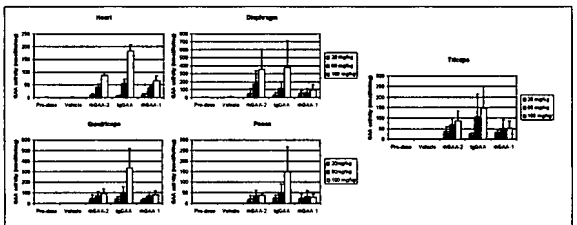

Figure 3:
Enzyme activity analysis of selected tissues from Pompe mice treated with tgGAA or one of two rhGAA preparations

Western Blotting

Western blot analysis was undertaken using a monoclonal antibody to GAA to determine the proteolytic processing status of GAA in the Pompe mouse tissues and to investigate lysosomal targeting. Both rhGAA and tgGAA are administered as 110kDa precursor molecules that are cleaved intracellularly into two mature forms of the enzyme (76 and 70kDa), via a 95kDa intermediate.

This analysis demonstrated that all enzyme preparations tested were processed appropriately in the tissue homogenates examined, suggesting that all enzymes are targeting correctly to the lysosomes within the tissues, including tgGAA.

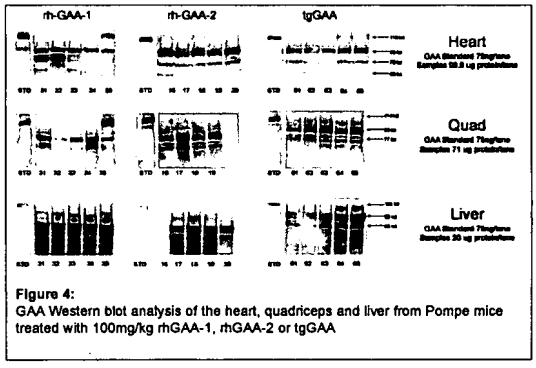

Figure 4:
GAA Western blot analysis of the heart, quadriceps and liver from Pompe mice treated with 100mg/kg rhGAA-1, rhGAA-2 or tgGAA

Conclusions

- Both tgGAA and rhGAA clear glycogen in a dose-dependent manner from the tissues of Pompe mice. Under the conditions of this study, the reduction in glycogen was more significant in mice treated with the rhGAA produced in CHO cells.
- Higher levels of GAA activity were detected in the tissues of mice treated with tgGAA, and Western blot data demonstrated that all enzymes appear to be processed correctly and targeted to the lysosomes. This suggests that tgGAA may be preferentially targeting to cell types other than glycogen containing myocytes within the tissues.
- Both CHO cell preparations demonstrated similar glycogen clearance and enzyme activity in the tissues examined.
- The results obtained from in vitro fibroblast uptake studies (see Poster #2691) do not predict *in vivo* efficacy, as shown here. This illustrates the significant impact of competing mechanisms of uptake present in a multi-cellular system.
- The murine data presented here may not predict the results obtained in the human clinical trials since the human disease genotypes, and phenotypes are quite different.

Acknowledgements

Department of Pharmacology and Toxicology
Joseph Serriello
Silvana Romano
Nancy Moore
Nikkol Atwell

Department of Pathology

Preclinical Biology
Michael O'Callaghan